ADVANCEMENTS IN RUMINANT TRACE MINERAL NUTRITION

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INTRODUCTION

Major advances in the field of trace mineral nutrition occurred in the twenty century. All of the trace minerals recognized as being important in ruminant nutrition were first shown to be essential in the 1900's with the exception of iron. Between 1920 and 1960 naturally occurring deficiencies of iodine, copper, cobalt, manganese, selenium, and zinc were first reported in cattle. Today it is common to supplement a number of trace minerals to ruminant diets to ensure that requirements are met. However, recommendations from textbooks and extension publications from the early to mid 1900's suggests that minimal supplementation of trace minerals occurred during this time. In the 20th edition of Feeds and Feeding published in 1936, Dr. F. B. Morrison stated "In addition to Ca, P, and common salt, iodine and also iron and copper must sometimes be considered in the feeding of dairy cattle. Sufficient amounts of the other essential minerals are supplied by any ordinary ration, as far as is known". Dr. Morrison stated in his 1956 edition of Feeds and Feeding "Except in the areas where there is a deficiency of one of the trace minerals - iodine, cobalt, copper, iron, or manganese - there is no benefit from feeding a trace mineral supplement, or in using trace mineralized salt instead of common salt.

TRACE MINERAL ANTAGONISTS

A potent interaction between copper, molybdenum, and sulfate was described in Australia by Dick in the early 1950's (McDowell, 2003). Since that time it has become clear that mineral antagonists can greatly affect absorption, and thus requirements of certain trace minerals. Some antagonist can react with certain trace minerals to form insoluble complexes or complexes that are too large to be absorbed. Antagonistic interactions can also occur between trace minerals due to competition for a transporter involved in absorption.

Sulfur

Sulfur, at levels (0.20-0.50%) typically found in ruminant diets, can be an important copper antagonist. In recent years, the concentration of sulfur in ruminant diets has increased due to increased use of by-products, such as dried distillers grains with solubles and corn gluten feed, that are high in sulfur. Sulfur in the form of sulfide is believed to reduce copper absorption due to the formation of insoluble copper sulfide in the gut (Suttle, 1974). In the rumen environment sulfide is produced from reduction of inorganic sulfur sources and degradation of sulfur amino acids. Increasing dietary sulfur in the inorganic (sulfate) or organic (methionine) form from a low level (0.10) to 0.40% reduced copper bioavailability in sheep by 30-56% (Suttle, 1974). Although sulfur is more likely to be of practical importance in cattle fed diets marginal in copper, high

sulfur also reduces liver copper stores in cattle supplemented with normal concentrations of copper (Spears et al., 2011).

Molybdenum-Sulfur-Copper

A very important three-way interaction occurs between molybdenum, sulfur, and copper in ruminants. This interaction can occur with concentrations of molybdenum and sulfur that often occur naturally in feedstuffs, and this complex interaction is centered around the formation of thiomolybdates (mono-, di-, tri-, and tetrathiomolybdates) in the rumen (Gooneratne et al., 1989). Thiomolybdates are formed by molybdate reacting with sulfide, produced during ruminal fermentation. Thiomolybdates associated with solid rumen digesta (bacteria, protozoa, and undigested feed particles) form insoluble complexes with copper that do not release copper even under acidic conditions (Allen and Gawthorne, 1987). In addition to reducing copper absorption certain thiomolybdates may be absorbed and produce systemic effects on copper metabolism, such as increasing biliary excretion of copper from liver stores, and removal of copper from copper containing enzymes (Suttle, 1991).

When dietary sulfur is low (0.10%) molybdenum has little or no affect on copper bioavailability (Suttle, 1975). However, the addition of 0.30% sulfur and 4 mg molybdenum/kg of diet to a basal diet containing 0.10% sulfur and 0.50 mg molybdenum/kg of diet reduced copper bioavailability by 40-70% in sheep (Suttle, 1975).

Iron

It is not uncommon for ruminant diets to exceed iron requirements by 5-fold or greater. Hays and silages are highly variable but can exceed 1,000 mg Fe/kg. Many by-product feeds are also fairly high in iron. Use of phosphate supplements greatly increases dietary iron, as commercial monocalcium and dicalcium phosphates contain approximately 10,000 mg Fe/kg. Cattle grazing pastures may be exposed to high iron through forage and/or soil ingestion.

A number of studies have indicated that high dietary iron is a copper antagonist. The addition of high iron (250 - 1,000 mg Fe/kg) from ferrous carbonate (Bremner et al., 1987) or ferrous sulfate (Mullis et al.,2003) to cattle diets greatly reduces copper status. Iron is also a manganese antagonist. It has been demonstrated the high dietary iron down regulates divalent metal transporter 1 (DMT1), the major transport protein involved in iron absorption by intestinal cells (Hansen et al., 2010). Although DMT1 is regulated by iron, it also transports other metals including manganese (Miret et al., 2003) High dietary iron may reduce absorption of manganese by competition for DMT1 as well as reducing the actual amount of transport protein present in the intestine.

Little is known regarding bioavailability of iron naturally present in feedstuffs. High dietary iron may not cause adverse effects on animal performance or health if the iron present in the diet is of low bioavailability. However, if iron present in feeds is fairly

bioavailable, adverse effects of high iron are more likely to be seen. When high iron concentrations are detected in forages it is unclear how much of the iron is naturally present in the forage and how much is due to soil contamination. In most soils iron is extremely high. Iron in soil is generally of low solubility and probably very poorly absorbed when ingested by cattle. Recent research suggests that acid conditions occurring during the fermentation of silage or haylage greatly increases the bioavailability of iron from soil contamination (Hansen and Spears, 2009).

Legumes may also represent an important source of available iron in ruminant diets. Much of the iron in soybeans has been shown to be present in the form of ferritin. Studies have indicated that iron in soybean ferritin is absorbed as well as ferrous sulfate in humans (Lonnerdal et al., 2006). If ferritin is a major form of iron in other legumes, such as alfalfa and clovers, this may represent a highly bioavailable form of iron in ruminant diets.

INORGANIC AND ORGANIC TRACE MINERAL SOURCES

Inorganic trace mineral sources (primarily sulfate and oxide forms) started being supplemented to cattle to a limited extent in the 1930's, and are widely used today. Supplementation of inorganic trace minerals has been effective in correcting as well as preventing trace mineral deficiencies in cattle. However, in the presence of certain antagonist, bioavailability of inorganic trace mineral sources can be low. Various feed-grade sources of a particular metal (oxide, sulfate, etc.) can also differ in purity, and other factors that can affect bioavailability of the mineral.

Organic trace minerals are complexed or chelated to organic ligands (amino acids or polysaccarides). Use of organic trace mineral sources to replace at least a portion of supplemental inorganic trace minerals has increased greatly in the past twenty years. In theory the covalent bonds formed between the metal and ligand (s) should allow organic trace minerals to resist many of the interactions encountered by inorganic trace minerals. Organic sources of trace minerals have been found to be more bioavailable than inorganic sources in some studies (Wedekind et al., 1992; Hansen et al., 2008). However, results have been variable and many studies have reported no differences in bioavailability between inorganic and organic sources (Cao et al., 2000; Guo et al., 2001).

HYDROXY TRACE MINERALS

Hydroxy trace minerals represent a new category of trace minerals. Basic copper chloride ($Cu_2(OH)_3Cl$) was introduced in 1995. Zinc hydroxychloride ($Zn_5(OH)_8Cl_2$) and manganese hydroxychloride ($Mn_2(OH)_3Cl$) were introduced to the market in 2012. In contrasts to sulfates where the metal is bound to sulfate via weak ionic bonds, the metals in hydroxy trace minerals are covalently bonded to multiple hydroxy groups. Hydroxy trace minerals are relatively insoluble in water but become soluble under acidic conditions typical of those found in the abomasum of ruminants. The low solubility in water results in hydroxy trace minerals being non hygroscopic and less reactive in feeds

and premixes than sulfates, resulting in improved vitamin stability (Lu et al., 2010) and less oxidation of lipids (Miles et al., 1998).

Many of the interactions among trace minerals in ruminants occur in the rumen environment. One advantage of hydroxy minerals is their ability to by-pass the rumen, thus minimizing interactions that normally occur in the rumen. Their ability to avoid interactions in the rumen can at least partially explain the higher bioavailability of hydroxy minerals compared with sulfate sources that have been reported in cattle. Bioavailability of basic copper chloride relative to copper sulfate was 132 to 196% in cattle fed diets high in sulfur and molybdenum (Spears et al., 2004). They postulated that the low solubility of basic copper chloride under the weakly acidic rumen conditions prevented the formation of stable copper complexes in the rumen, thus increasing the absorption of copper in the small intestine. Steers supplemented with zinc hydroxychloride had a greater ($P< 0.01$) apparent absorption (19.8 vs. 9.9%) and retention of zinc (77 vs. 35 mg/day) than steers receiving zinc sulfate (Shaeffer, 2006).

MECHANISMS OF TRACE MINERAL ABSORPTION

Perhaps the most significant advancement in trace minerals in recent years relates to research that has advanced our understanding of trace mineral absorption and homeostasis. A number of transporters have been characterized that are involved in cellular uptake and export of iron (De Domenico et al., 2008), copper (Kim et al., 2008), and zinc (Cousins et al., 2006). Transporters and proteins involved in the regulation of iron (Hansen et al., 2010) and copper (Fry et al., 2013) have been characterized in cattle.

A review by Kim et al (2008) discusses in detail the mechanisms involved in copper absorption and regulation in the body. Copper transporter 1(Ctr1) is the major transporter involved in cellular uptake of copper by intestinal and other mammalian cells. This transporter is a high-affinity copper import protein that is specific for Cu^{+1}, and a reductase apparently is involved in reducing Cu^{+2} to Cu^{+1}. Free copper in the cell must be controlled to prevent oxidative damage to cells. Therefore, copper imported into enterocytes and other cells is trafficked to specific destinations within the cell bound to small molecules known as copper chaperones. Three different chaperones have been identified that deliver copper to specific targets. Copper chaperone protein (CCS) transports copper to Cu/Zn superoxide dismutase in the cytosol. A second chaperone protein, Cox 17, transports copper to proteins in the mitochondrial that transfer copper to cytochrome c oxidase in the inner mitochondrial membrane. The copper chaperone Atox 1 transports copper to copper ATPases in the trans-golgi network. Copper ATPase 7A is involved in actively transporting copper from intestinal cells into the blood, and also in transporting copper to cuproenzymes that are secreted from the golgi network into the plasma membrane. A second copper ATPase, ATPase 7B is expressed primarily in the liver, where it is important in secretion of copper from the liver via biliary excretion and incorporation into ceruloplasmin.

Two families of zinc transporters have been identified. The Zip family transporters promote zinc import into cells, while the ZnT proteins are involved in zinc export from cells or influx into intracellular vesicles (Cousins et al., 2006). Several different Zip and ZnT transporters have been identified. Zip4 is considered to be the major intestinal zinc import transporter because a genetic mutation of this transporter results in severe zinc deficiency in humans and cattle due to impaired zinc absorption (Yuzbasiyan-Gurkan and Bartlett, 2006). It is well documented that zinc absorption is homeostatically regulated and expression of a number of zinc transporters are affected by level of dietary zinc.

Iron absorption from the small intestine is tightly regulated due to the limited ability of the body to excrete absorbed iron. It is critical that absorbed iron be bound because free iron causes generation of reactive oxygen species that lead to tissue oxidative damage. Absorption of iron occurs primarily in the duodenum and upper jejunum. As mentioned earlier DMT1 is the major transporter that imports iron across the enterocyte apical membrane (De Domenico et al., 2008). DMT1 is specific for Fe^{+2} and a ferrireductase on the apical surface of enterocytes reduces Fe^{+3} prior to transport. Iron imported by DMT1 can be stored in the enterocyte in ferritin or be exported into plasma by ferroportin, a basal membrane iron transporter (De Domenico et al., 2008). On the basolateral membrane iron must be oxidized to Fe^{+3} by hephaestin, a copper containing ferroxidase, for iron export by ferroportin. Iron exported from the intestine by ferroportin must be in the Fe^{+3} state to bind transferrin, the iron transport protein in plasma. Iron stored in ferritin can be lost from the body during normal turnover of enterocytes or released from ferritin for export into the bloodstream via ferroportin. Hepcidin, a small peptide hormone produced in the liver and secreted into plasma, is important in regulating iron absorption (De Domenico et al., 2008). Expression of hepcidin is regulated by liver iron stores. Hepcidin signals the small intestine to down regulate iron absorption.

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